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Final Report
R-88-05-0303

Optimizing a Tissue Culture System to Produce
Disease-Free, Nuclear Stock for Potato Seed and
Development a Tissue culture Based Model for
Saskatchewan Seed Production

Funded by
The Agriculture Development Fund

**Abstract
of
Optimizing a Tissue Culture System to Produce Disease-Free,
Nuclear Stock for Potato Seed and Develop a Tissue Culture
Based Model for Saskatchewan Seed Production.**

Contract # R-88-05-0303

**Funded by;
Saskatchewan Agriculture - Agriculture Development Fund**

**Prepared by;
Kenneth R.D. Friesen
September 27, 1990**

The potato (Solanum tuberosum) is an import crop worldwide. Canada is a major exporter of potato 'seed'. Because potatoes are propagated vegetatively diseases accumulate in the tuber tissues which cause reduced yields. Disease-free potato 'seed' can be produced through the use of plant tissue culture. Starting with meristem culture (Wilson 1986) disease-free tissue can be isolated. Combined with micropropagation techniques (Tovar et al 1985) many thousands of disease-free plants can be produced. By growing these plants in an insect proof greenhouse, disease-free nuclear 'class' minitubers can be produced.

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**Summary Report
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As a consequence of this form of propagation, diseases (primarily viruses and viroids) accumulate. Diseases contracted in one year are passed on to the next 'generation' through the tissue of the tuber. Each seasons crop adds to the concentration of disease causing organisms. Loss of vigour, yield reductions and loss of quality (size, shape and appearance) result.

Common disease organisms found in potato tubers are the viruses A, Y, M, S and X, Potato Leaf Roll virus, Spindle Tuber viroid, Bacterial Ring-rot (Corynebacterium sepedonicum) and Blackleg (Erwinia carotovora). Potato viruses A and Y produce distinctive symptoms while X and Potato Leaf Roll viruses have several strains which vary in severity and symptoms. M and S are

latent and produce few if any external symptoms. Virus A can cause a 40% reduction in yield (Hooker 1981), X by 15 - 30% and S causing no significant decline (Mellor and Stace-Smith 1987). The degree of symptom expression does not reflect the effect on yield. X and S combined can cause yield reduction by 20 - 35% (Mellor and Stace-Smith 1987).

To combat the spread of these diseases and to improve production, stringent testing and inspection regulations for the production of 'seed' potato tubers have been introduced in many potato producing nations. This combined with international plant quarantine regulations serve to reduce the spread of these infectious diseases.

Testing involves visual field inspection, indicator plants, ELISA, cDNA probes, electrophoresis, and both florescent and electron microscopy.

A second defence against the spread of these diseases is the production of 'disease-free' 'seed'. In the past this was accomplished by heat treatment of cuttings taken from sprouts. Tip cuttings taken from the heat treated shoots were rooted. The resultant rooted plants were grown in insect proof screened beds to produce pre-Elite tubers. From these tubers subsequent classes of 'seed' tubers were derived. These techniques helped to reduce the load of viruses present in the tissues but were slow. Five years or more would be required to build up a sufficient stock of certified 'seed'.

In 1957 techniques to grow potato under in vitro conditions

were developed (Kassanis 1957). The use of this technology enabled the production of 'disease free' plants through meristem culture (Mellor and Stace-Smith 1977). This involves the isolation of a 0.2 - 0.5 mm meristem from a heat treated shoot. This meristem is essentially free of virus particles. Although other plant tissues can be used, meristem culture produces a plant in the least amount time and with less risk of somaclonal variation or mutation (Mellor and Stace-Smith).

When combined with micropropagation techniques the subsequent rate of increase per year far exceeds that of the traditional methods. The same quantity of certified 'seed' can be produced in 2 years, depending on the micropropagation scheme selected. The entire increase of disease-free plants to produce Elite or lower classes of 'seed' may be accomplished by micropropagation. Other production schemes require only a few disease-free plants which are subsequently increased in the field using traditional methods (Mosely and Perrigan 1985, Matthews et al 1986).

Micropropagation techniques are varied. The methods used fall into 3 broad areas with many variations within each group. Shoots are derived from either meristem culture or by the initiation of axillary shoot formation of a sterile node taken from the source plant. The serial cutting method (George et al 1986, Hussey and Stacey 1981, Matthews et al 1986, Wilson et al 1986) sub-divides the initiating shoot into internodal pieces (one node per segment). There can be anywhere from 7 to 12 nodes per shoot. These nodes are then inoculated into a proliferation medium and again grown into

single shoots. This process continues with subculturing occurring every 4 to 6 weeks. The derived shoots are removed from culture and rooted either in vitro or in peat plugs prior to field transplanting.

Another method of micropropagation involves 'layering' techniques (Goodwin and Adisarwanto 1980, Goodwin et al 1980a, Goodwin et al 1980b). This process stimulates the development of axillary shoots along the length of the initiating shoot. Agitated liquid media are used. Resultant shoots are separated and subcultured to stimulate the development of axillary shoots without any further division into internodal segments. Eventually the 'clumps' of shoots are separated and placed into a rooting medium.

The third method of micropropagating potato is termed in vitro tuberization (Abbott and Belcher 1986, Tovar et al 1985, Estrada et al 1986, Wang and Hu 1982). Shoots derived from meristems are proliferated by either layering or by serial cutting and then exposed to media containing high concentrations of sucrose, cytokinins and other additives. Together with decreased lighting, these media promote the formation of microtubers of less than 1.0 g each. These tubers can be used for the production of plantlets under field or greenhouse conditions, for sterile long term storage or in breeding programs. They are also well suited for international exchange of plant material.

From the results of the experiments, described in the technical report, a schedule for the production of disease-free 'nuclear' class potato minitubers has been derived.

Year 1

- January: Pot superior tubers of the desired cultivars.
- February: Remove cuttings from the mother plants and give heat treatment for 3 weeks. Test both mother plant and heat treated cuttings for disease organisms; Viruses X, S, A, M, and PLRV with ELISA (Clark and Adams 1977), Bacterial Ring Rot with monoclonal immunofluorescence and gram stain, Blackleg with α -Methyl Glucoside Agar (Phillips 1977), and PSTV with Dot Blot (Nucleic Acid) Hybridization (Owens and Diener 1981). Initiate cultures using the technique devised by Wilson (Wilson et al 1986).
- May: Remove initiated meristems. Cut into internodal segments and subculture in the proliferation media devised by Tovar (Tovar et al 1985).
- June: Subculture proliferating shoots every 30 - 40 days. Randomly test for disease organisms as described above.
- September: Remove proliferating shoots with 6 - 8 nodes from culture and pot in trays 4" apart. Use a soilless potting mix supplemented with 11-48-0 or similar fertilizer at label rates (Jim Littel personal communication). Place trays in an insect proof greenhouse with regular watering and weekly feedings of 100:200:200 (ppm) N:P₂O₅:K₂O initially, followed by 200:200:200 (ppm) during tuber set and

sizing and returning to 100:200:200 (ppm) for final sizing and senescence (Anonymous). This could be facilitated by automated watering and a fertilizer injection system. Temperature should be between 22 and 25°C. Randomly test leaf tissue for disease organisms as described above.

December: Turn off watering 1 week before harvest. Harvest early cultivars, sort, wash, bag and label minitubers. Randomly test tuber tissue for disease organisms as described above. Minitubers should be stored at 15°C for 2 weeks at 95% relative humidity then at 4 - 5°C at 95% relative humidity till ready for distribution.

Pot second planting of shoots for production of minitubers as described above.

Year 2

January: Harvest late cultivars as described above. Pot second planting of shoots for production of minitubers. Sprout new lines of potatoes for Year 3 minitubers.

February: Heat treat and test mother plants and initiate meristems as described above.

March: Harvest second planting of minitubers as described above.

April: Begin distribution of minitubers to growers in late April.

Sanitation and random disease testing are vital to the program. This will protect against accidental infestation by disease carrying insects and infection with disease. Should disease be indicated, the random testing at all levels of production will aide in the isolation of the causal agents.

Tissue culture conditions must be aseptic and all greenhouse equipment and facilities must be cleaned and disinfected prior to use. To this end quaternary ammonium and hot water should be used. Pesticides to prevent and control insects and mites are essential.

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1. Introduction

The potato (Solanum tuberosum) is the most important horticultural food crop worldwide (Bajaj 1987). Production worldwide in 1986 was 308,548,000 metric tons. Canadian production the same year was 2,850,000 metric tons (I.E.S.A. 1988). Although Canada's total production is low, it is a major potato seed exporting nation (Van Der Zaag 1986). Saskatchewan production figures for 1989 were 327,040 cwt table grade and 45,470 cwt 'seed'. This was short of 1990 'seed' requirement projections for 1990 (Prairie Potato Council 1990).

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2. Technical Report - Methods and Materials

Purpose: To develop a tissue culture based system for disease freeing and proliferation of potato plants. These plants will be used to produce disease-free nuclear potato 'seed' tubers for the potato seed industry.

Method:

Mother Plant Material Preparation:

Nuclear potato 'seed' tubers of 5 cultivars were obtained from Dr. N. Wright, Agriculture Canada and the British Columbia Certified Seed Grower's Association in November 1988. The tubers were stored at 10°C in the dark and removed as required.

Tubers were sprouted and used as parent plant material. The following procedure was used:

Tubers were washed and submerged in a 10% v/v solution of domestic bleach (6% sodium hypochlorite) with a few drops of Tween-20 for 10 minutes. They were then rinsed in deionized distilled water and cut in half; the apical half was potted while the basal end was suberized, dusted with 'captan' and stored at 10°C.

The tuber halves were potted 3 inches deep and apical end up in 10 inch plastic pots using 40 - 60 mesh washed quartz sand as the substrate. The sand had been sterilized by autoclaving in the pot at 120°C and 104 Kpa for 1 hour. The pots were placed in a 'Convicon' growth chamber at 20°C with 16 hour day-length under florescent and incandescent lighting.

Experiment 1: The effect of medium formulation and length of tuber storage on initiation.

Purpose: To determine an optimum physiological state and medium for initiation.

Method:

Tubers were removed from storage after 0 (Fall), 60 (Winter) and 150 (Spring) days in storage. Source plants and meristems were prepared and collected as described above. Five cultivars were used; Russet Burbank, Norland, Yukon Gold, Raritan and Norgold Russet.

Explant isolation:

When the plants were grown to full size but prior to flowering (the time required varied with the length of time the tuber was in storage), internodal cuttings were taken. The leaves and petioles were removed by cutting with a scalpel.

Internodal pieces were surface sterilized in a 10% v/v solution of domestic bleach (6% sodium hypochlorite) with a few drops of Tween-20 for 15 minutes. They were then rinsed with 3, 5 minute washes in sterile deionized distilled water.

Meristems (0.3 mm long and comprising the meristematic dome and two leaf primordia) were removed by dissection from the nodes and placed in the appropriate medium.

Three initiation techniques were examined as described by U.E. Wilson (Wilson et al 1986), P. Wang and C. Hu (Wang and Hu 1982) and a F.A.O. technical publication by R.A.T. George (George 1986) (see Appendix 1). These techniques were selected as representative

of the many techniques presently used in production of micropropagated potato. All media were prepared with deionized distilled water, dispensed into the appropriate culture vessel and sterilized by autoclaving at 120°C and 104 Kpa for 20 minutes. Ten meristems of each cultivar were used for each technique.

The techniques described by the F.A.O. and by Wang and Hu used 8 ml of agar solidified media in 18 x 150 mm 'Kimax' culture tubes and capped with 'Bacti-Capall' closures. Inoculated cultures were placed in light racks and exposed to full light of 4 florescent 'Gro-lux' tubes for 16 hour days and maintained at 21 - 25°C. The technique described by Wilson is a liquid medium designed to be agitated. Ten mls of medium were dispensed into 22 x 150 mm 'Kimax' culture tubes and loosely closed with screw caps. Inoculated cultures were placed well spaced into test-tube racks and agitated on a reciprocal shaker at 60 rpm under low illumination of 2 florescent lamps and maintained at 21 - 25°C.

The stage of meristem development was recorded with a numerical code for each meristem after 100 days in culture. The following rating system was used:

- 0 = dead
- 1 = alive but no development
- 2 = alive with 1 expanded leaf
- 3 = alive with 2 expanded leaves
- 4 = alive with more than 2 leaves but no visible shoot
- 5 = alive with more than 2 leaves and elongated shoot.

The percentage of usable meristems (encoded 4 and 5) was calculated for each cultivar for each of the 3 lengths of tuber storage.

Experiment 2: Proliferation.

Purpose: To identify a technique to proliferate in vitro 'disease free' potato plantlets derived from meristem culture.

Method:

Plantlets of the cultivars Russet Burbank and Norland were derived from meristems of tubers stored for 0 and 60 days. Meristems were initiated using the technique devised by Wilson (Wilson et. al. 1986). Plantlets, derived from one meristem were cut into internodal segments (one node per segment). Each segment was inoculated into either the proliferation medium devised by Wilson (Wilson et al 1986) or by Tovar (Tovar et al 1985) (see Appendix 1 Table 2).

Eight mls of each media was dispensed into 18 x 120 mm 'Kimax' culture tubes. After inoculation cultures were placed in light racks with 4 florescent lamps shaded by 50% with cheese cloth. They were exposed to 16 hour day-lengths and maintained at 21 - 25°C for 6 weeks. Cultures were subcultured at the end of 6 weeks by removing each new shoot and cutting it into internodal segments and reculturing onto fresh media. The number of nodes obtain from 10 plantlets derived from the original meristem was recorded.

Experiment 3: In vitro microtuber production.

Purpose: To determine the rates of production of in vitro microtubers from micropropagated potato.

Methods:

Plantlets of the cultivar Norland were derived from meristems

using the technique by Wilson previously described. Plantlets were proliferated using the technique of Tovar (Tovar et al 1982) used in experiment 2.

Two techniques were examined to produce microtubers. One method followed the technique of Tovar. This involved removing both leaves and roots from micropropagated plantlets and laying them horizontally individually into 250 ml erlenmeyer flasks. The flasks contained a liquid proliferation medium (see Appendix 1 Table 3) and were sealed with a sterile cotton plug. After inoculation the culture vessels were placed in a reciprocal shaker and agitated at 60 rpm. The cultures were maintained at 21 - 25°C under 2 florescent lamps with 16 hour daylength for 3 weeks. After 3 weeks the liquid proliferation medium was decanted and replaced with a liquid tuber induction medium (see Appendix 1 Table 3) and agitated for 30 days under darkened conditions at 21 - 25°C.

A second method to produce microtubers involved leaving the micropropagating plantlets in the medium devised by Tovar for an additional 6 weeks past the time required for proliferation under darkened conditions at 21 - 25°C.

Data on the number of microtubers produced and their weight was collected after the required time.

Experiment 4: Ex vitro minituber formation.

Purpose: To determine the number and size range of greenhouse grown, disease-free minitubers.

Methods: Micropropagated plantlets of the cultivars Russet Burbank and Norland were removed from culture and potted in individual 4" plastic pots in a 30 m² greenhouse. The plantlets were covered with 'Terra-Lite' soilless potting mix so that only the top 2 leaves were exposed. After potting the soil-mix was wetted with a solution of 20-20-20 liquid fertilizer at label rates. The pots were covered with tight-fitting clear polyethylene bags to maintain a humid environment while the plantlets hardened. After 1 week the bags were removed. Three weeks after potting or when plantlets were 6" high, B-Nine-SP (Uniroyal) was applied at 7500 ppm following label directions.

The greenhouse was completely screened to prevent entry of insects from outside or between greenhouses. Natural lighting was supplemented with 6 sodium vapour lamps to provide 16 hour daylengths. Automated watering was used to prevent uneven moisture levels, while automated, thermostatically controlled vent openers maintained greenhouse temperatures generally between 21 and 35°C. Data was collected on the number and weight of each tuber from each potted plant after 4 months.

3. Results

Experiment 1: The effect of medium formulation and length of tuber storage on initiation.

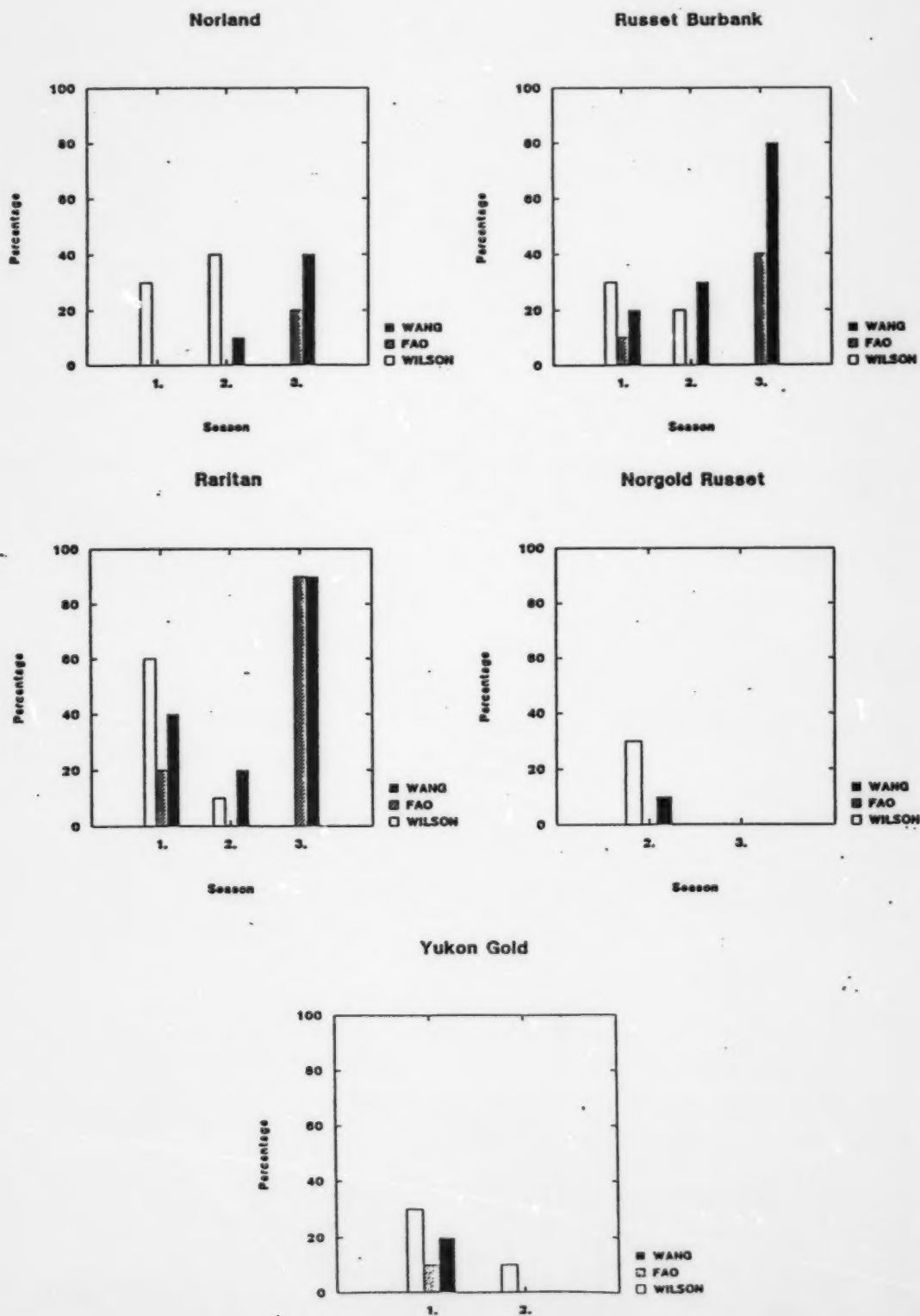
Results from the experiments to identify a technique to initiate in vitro potato plants from meristems are presented in Figure 3.1 in terms of percentage of useable shoots. Means of cultivars and treatments are given in Appendix 2.

None of the 3 media used gave consistent results with meristems collected from either fall, winter or spring tubers among any of the of the cultivars used. Overall the medium devised by Wilson gave the best results with fall and winter meristems while that devised by Wang and Hu gave the best results for spring meristems. Shoots derived from meristems initiated on Wilson were more vigorous with longer shoots and more nodes than those derived from the FAO or Wang and Hu media.

Provided that the appropriate medium is used for initiation, meristems can be collected and initiated from tubers at fall, winter or spring. This is important because it removes restrictions on the timing of initiation of meristems. Year-round initiation of cultivars is possible thus eliminating breaks in production scheduling.

The low percentage of usable shoots is a reflection of the small size of the meristems used. Smaller meristems are less likely to harbour disease organisms than larger meristems. Smaller meristems are also less likely to initiate new growth to form a shoot (Mellor and Stace-Smith 1987). However, only shoots derived

Figure 2.1 Percentage of Usable Shoots Derived From Three Initiation Media and Tubers From Three Storage Intervals.



from one meristem of each cultivar should be used for proliferation.

Both Norgold Russet and Yukon Gold failed to produce vigorous mother plants reliably. As a result no meristems were collected from fall collected Norgold Russet and spring collected Yukon Gold. These cultivars also proved to be difficult to initiate. Once initiated resultant shoots were vigorous.

Observations made throughout the experiment indicate that an acceptable number of meristems develop into usable sized shoots after 75 days although more are available after 100 days.

Experiment 2: Proliferation.

No shoots developed on the proliferation medium devised by Wilson (Wilson et al 1986). Preliminary experiments had indicated that this medium was acceptable. No explanation for its failure to generate shoots in this experiment could be found.

Proliferation data of shoots on the medium devised by Tovar are in table 3.1 below.

TABLE 3.1 Proliferation In Number of Nodes per Shoot for Subculture

CULTIVAR	SOURCE PLANT	MEAN NUMBER OF NODES	STANDARD DEVIATION
NORLAND	FALL	8.4	8.4
NORLAND	WINTER	10.4	6.0
RUSSET BURBANK	FALL	11.1	6.5
RUSSET BURBANK	WINTER	8.1	5.5

There was no significant difference within a cultivar between plantlets derived from fall or winter source plants. Data for each cultivar was combined to obtain general means. These are presented in Table 3.2.

TABLE 3.2 Proliferation In Number of Nodes per Shoot for Subculture; Combined Data.

CULTIVAR	MEAN NUMBER OF NODES	STANDARD DEVIATION
NORLAND	9.4	7.3
RUSSET BURBANK	9.6	6.1

There was no significant difference between cultivars. Although there was no other treatment with which to compare, the

results show an acceptable level of proliferation for both cultivars. From a single meristem initiated in 75 days and subcultured and proliferated every 42 days, it would be possible to generate 60,956,894 plantlets in 369 days. This far exceeds the requirements of the Saskatchewan seed potato industry. The results also indicate that plantlets derived from tubers without storage do not differ in rates of proliferation than those derived from tubers stored until winter. There are no restrictions on the rate of proliferation nor the media used for different cultivars or physiologic age of tuber from which mother plants were derived.

Experiment 3: In vitro microtuber production.

Results of the experiment to produce microtubers using the complete Tovar protocol or by allowing the plantlet proliferating in the Tovar proliferation medium to age and dry-down, are found in Table 3.3.

Table 3.3 Means of Microtuber Production.

TECHNIQUE	MEAN NUMBER OF TUBERS	sd	MEAN WEIGHT per TUBER gm.	sd gm.	MEAN TOTAL TUBER WEIGHT gm.	sd gm.
TOVAR COMPLETE	3.8	2.6	0.1	0.1	0.4	0.2
TOVAR DRY-DOWN	3.3	3.2	0.1	0.1	0.3	0.2

There was no significant difference between the two methods to produce microtubers. Both treatments produced small tubers of similar size and shape. Allowing the plantlets to use up their nutrient medium, age and dry-down is more time efficient (12 weeks) and as effective as using the lengthier (13 weeks) and involved process devised by described by Tovar.

Microtubers have been used as 'nuclear' class seed for greenhouse and field plantings but their use is limited by their long dormancy induced by in vitro conditions. They can be useful for sterile storage of genotypes because they resist somaclonal variation, can be easily maintained under sterile conditions and occupy little space. This method of storage is ideal for a tissue culture facility where time is at a premium. Less time spent on maintenance of long term cultures frees time for new cultivar initiation, disease freeing procedures and proliferation of

plantlets. It is doubtful whether this technology would be useful for the Saskatchewan 'seed' potato industry at this time.

Experiment 4 Ex vitro minituber formation.

Results of the experiment to produce minitubers from micropropagated potato plantlets are presented in Table 3.4.

Table 3.4 Number and Weight of Minitubers.

CULTIVAR	MEAN NUMBER OF TUBERS	sd	MEAN WEIGHT OF TUBERS gm.	sd gm.	MEAN TOTAL WEIGHT OF TUBERS gm.	sd gm.
NORLAND	2.4	1.0	9.3	6.8	22.1	11.2
RUSSET BURBANK	3.4	1.6	6.5	7.4	22.6	11.4

There was no significant difference between cultivars although it appears that Russet Burbank produces more smaller minitubers than Norland. Total yield in weight is the same however. Not all minitubers produced will be of an acceptable size consequently a breakdown of total yield was determined. Table 3.5 shows the range of minituber sizes and their percentage within the total yield of each cultivar.

Table 3.5 Size Classes and Percentage of Minituber Yield.

CULTIVAR	0 - 0.9 gm.	sd %	1.0 - 4.9 gm.	sd %	5.0 - 9.9 gm.	sd %	10.0 -14.9 gm.	sd %
NORLAND	8.9	21.1	16.2	25.6	33.4	32.3	20.3	25.9
RUSSET BURBANK	29.6	45.1	35.1	42.2	18.6	28.2	19.6	26.4

Table 3.5 continued.

CULTIVAR	15.0 - 19.9 gm.	sd %	20.0 + gm.	sd %	USEABLE SIZE 5.0 + gm.	sd %
NORLAND	15.3	28.4	8.2	21.6	77.1	27.9
RUSSET BURBANK	3.6	28.6	8.5	16.6	50.3	37.2

Norland produces fewer minitubers overall but 77.1% are of a usable size (5.0 - 20.0 or greater grams). This corresponds to a yield of approximately 1,850 usable minitubers per 1,000 plants. This is significantly different from Russet Burbank which produces more tubers overall with only 50.3% being of a usable size. This corresponds approximately to 1,710 usable minitubers per 1,000 plants. Methods to increase the number of usable Russet Burbank minitubers should be investigated. This could include altering the amount or formulation of fertilizer and the application schedule. The variability of the results should also be taken in consideration when planning production schedules.

Conclusions

From the results of the experiments described, the following schedule for the production of disease-free 'nuclear' class potato minitubers is derived.

Year 1

January: Pot superior tubers of the desired cultivars.

February: Remove cuttings from the mother plants and give heat treatment for 3 weeks. Test both mother plant and heat treated cuttings for disease organisms; Viruses X, S, A, M, and PLRV with ELISA (Clark and Adams 1977), Bacterial Ring Rot with monoclonal immunofluorescence and gram stain, Blackleg with α -Methyl Glucoside Agar (Phillips 1977), and PSTV with Dot Blot (Nucleic Acid) Hybridization (Owens and Diener 1981). Initiate cultures using the technique devised by Wilson (Wilson et al 1986).

May: Remove initiated meristems. Cut into internodal segments and subculture in the proliferation media devised by Tovar (Tovar et al 1985).

June: Subculture proliferating shoots every 30 - 40 days. Randomly test for disease organisms as described above.

September: Remove proliferating shoots with 6 - 8 nodes from culture and pot in trays 4" apart. Use a soilless potting mix supplemented with 11-48-0 or similar fertilizer at label rates (Jim Littel personal

communication). Place trays in an insect proof greenhouse with regular watering and weekly feedings of 100:200:200 (ppm) $N:P_2O_5:K_2O$ initially, followed by 200:200:200 (ppm) during tuber set and sizing and returning to 100:200:200 (ppm) for final sizing and senescence (Anonymous). This could be facilitated by automated watering and a fertilizer injection system. Temperature should be between 22 and 25°C. Randomly test leaf tissue for disease organisms as described above.

December: Turn off watering 1 week before harvest. Harvest early cultivars, sort, wash, bag and label minitubers. Randomly test tuber tissue for disease organisms as described above. Minitubers should be stored at 15°C for 2 weeks at 95% relative humidity then at 4 - 5°C at 95% relative humidity till ready for distribution.

Pot second planting of shoots for production of minitubers as described above.

Year 2

January: Harvest late cultivars as described above.

Pot second planting of shoots for production of minitubers. Sprout new lines of potatoes for Year 3 minitubers.

February: Heat treat and test mother plants and initiate meristems as described above.

March: Harvest second planting of minitubers as described above.

April: Begin distribution of minitubers to growers in late April.

Sanitation and random disease testing are vital to the program. This will protect against accidental infestation by disease carrying insects and infection with disease. Should disease be indicated, the random testing at all levels of production will aide in the isolation of the causal agents.

Tissue culture conditions must be aseptic and all greenhouse equipment and facilities must be cleaned and disinfected prior to use. To this end quaternary ammonium and hot water should be used. Pesticides to prevent and control insects and mites are essential.

Appendix 1 Table 1 Formulations of initiation media used in initiation experiment 1.

Compound	F A O ¹ (mg/l)	Wilson ² (mg/l)	W a n g ³ (mg/l)
MACRONUTRIENTS			
NH ₄ NO ₃	1650	1650	550
KNO ₃	1900	1900	633.3
CaCl ₂ ·2H ₂ O	440	440	146.7
MgSO ₄ ·7H ₂ O	500	370	123.3
KH ₂ PO ₄	170	170	56.7
FeSO ₄	27.8	27.8	9.3
MICRONUTRIENTS			
H ₃ BO ₄	1.0	6.2	2.1
MnSO ₄ ·4H ₂ O	0.5	22.3	7.4
KI	0.01	0.83	.277
CuSO ₄ ·5H ₂ O	0.03	0.025	.008
ZnSO ₄ ·7H ₂ O	1.0	8.6	2.87
AlCl ₃	0.03	-	-
NiCl ₂	0.03	-	-
Na ₂ EDTA	38.0	37.3	12.4
NaMoO ₄ ·2H ₂ O	-	0.25	.08
CoCl ₂	-	0.025	.008
VITAMINS			
Nicotinic Acid	1.0	0.5	.17
Thiamine HCl	1.0	0.1	.03
D ⁽⁺⁾ Ca-Pantothenate	0.5	-	-
Riboflavin	0.1	-	-
Biotin	1.0	-	-
Pyridoxine HCl	-	0.5	.17
Adenine Sulfate	80	-	-
myo-Inositol	100	100	33.3
Bacto-tryptone	-	-	1000
GROWTH REGULATORS			
Kinetin	-	-	1.0
p-Aminobenzoic Acid	1.0	-	-
Gibberellin	-	0.1	-
NAA	-	-	0.01
IBA	0.2	-	-
OTHER			
Sucrose	20000	30000	30000
Glycine	-	2.0	.67
Agar	7500	-	8000
pH	5.3-5.5	5.2-5.8	5.3

¹ George, R.A.T. 1986

² Wilson, U.E. et. al. 1986

³ Wang, P. and Hu, C. 1982

Appendix 1 Table 2. Formulations of proliferation media used in proliferation experiment 3.

Compound	T o v a r ¹ (mg/l)	W i l s o n ² (mg/l)
MACRONUTRIENTS		
NH ₄ NO ₃	1650	1650
KNO ₃	1900	1900
CaCl ₂ ·2H ₂ O	440	440
MgSO ₄ ·7H ₂ O	370	370
KH ₂ PO ₄	170	170
FeSO ₄	27.8	27.8
MICRONUTRIENTS		
H ₃ BO ₄	6.2	6.2
MnSO ₄ ·4H ₂ O	16.9	22.3
KI	0.83	0.83
CuSO ₄ ·5H ₂ O	0.025	0.025
ZnSO ₄ ·7H ₂ O	8.6	8.6
AlCl ₃	-	-
NiCl ₂	-	-
Na ₂ EDTA	37.3	37.3
NaMoO ₄ ·2H ₂ O	0.25	0.25
CoCl ₂	0.025	0.025
VITAMINS		
Nicotinic Acid	-	0.5
Thiamine HCl	0.4	0.1
D ⁽⁺⁾ Ca-Pantothanate	2.0	-
Riboflavin	-	-
Biotin	-	-
Pyridoxine HCl	-	0.5
Adenine Sulfate	-	-
myo-Inositol	100	100
Bacto-tryptone	-	-
GROWTH REGULATORS		
Kinetin	-	-
p-Aminobenzoic Acid	-	-
Gibberellin	0.25	0.1
NAA	0.01	-
IBA	-	-
OTHER		
Sucrose	30000	30000
Glycine	-	2.0
Agar	8000	7200
pH	5.4	5.2-5.8

¹ Tovar, P. et al 1986

² Wilson, U.E. et. al. 1986

Appendix 1 Table 3. Formulations of Tovar¹ media used in microtuber experiment 4.

Compound	L i q u i d Proliferation (mg/l)	Liquid Induction (mg/l)	Tuber
MACRONUTRIENTS			
NH ₄ NO ₃	1650	1650	
KNO ₃	1900	1900	
CaCl ₂ ·2H ₂ O	440	440	
MgSO ₄ ·7H ₂ O	370	370	
KH ₂ PO ₄	170	170	
FeSO ₄	27.8	27.8	
MICRONUTRIENTS			
H ₃ BO ₃	6.2	6.2	
MnSO ₄ ·4H ₂ O	16.9	16.9	
KI	0.83	0.83	
CuSO ₄ ·5H ₂ O	0.025	0.025	
ZnSO ₄ ·7H ₂ O	8.6	8.6	
Na ₂ EDTA	37.3	37.3	
NaMoO ₄ ·2H ₂ O	0.25	0.25	
CoCl ₂	0.025	0.025	
VITAMINS			
Thiamine HCl	0.4	0.4	
D ⁽⁺⁾ Ca-Pantothenate	2.0	-	
myo-Inositol	100	100	
GROWTH REGULATORS			
p-Aminobenzoic Acid	0.5	5.0	
Gibberellin	0.4	-	
NAA	0.01	-	
Chlorocholine chloride	-	500	
OTHER			
Sucrose	30000	80000	
pH	5.4	5.4	

¹ Tovar, P. et al 1986

Appendix 2.

Table 1.1 MEANS OF MERISTEM INITIATION ON THREE MEDIA AND FROM THREE STORAGE INTERVALS.

CULTIVAR	SEASON	MEDIUM	GROWTH 0-5*	sd
NORLAND	FALL	WILSON	3.4	1.2
NORLAND	FALL	FAO	3.7	0.6
NORLAND	FALL	WANG and HU	2.4	1.0
NORLAND	WINTER	WILSON	3.3	1.2
NORLAND	WINTER	FAO	1.3	0.5
NORLAND	WINTER	WANG and HU	2.4	1.1
NORLAND	SPRING	WILSON	0	0
NORLAND	SPRING	FAO	3.2	1.1
NORLAND	SPRING	WANG and HU	3.5	1.2
RUSSET BURBANK	FALL	WILSON	3.3	1.5
RUSSET BURBANK	FALL	FAO	2.5	0.9
RUSSET BURBANK	FALL	WANG and HU	2.7	0.9
RUSSET BURBANK	WINTER	WILSON	3.7	1.0
RUSSET BURBANK	WINTER	FAO	1.4	0.5
RUSSET BURBANK	WINTER	WANG and HU	3.3	0.8
RUSSET BURBANK	SPRING	WILSON	0	0
RUSSET BURBANK	SPRING	FAO	3.8	1.4
RUSSET BURBANK	SPRING	WANG and HU	4.0	0.0
RARITAN	FALL	WILSON	4.1	1.1
RARITAN	FALL	FAO	3.1	0.9
RARITAN	FALL	WANG and HU	2.8	1.1
RARITAN	WINTER	WILSON	3.1	0.9
RARITAN	WINTER	FAO	1.4	0.5
RARITAN	WINTER	WANG and HU	2.7	1.1
RARITAN	SPRING	WILSON	0	0
RARITAN	SPRING	FAO	4.7	0.5
RARITAN	SPRING	WANG and HU	3.5	1.6

APPENDIX 2 TABLE 1.1 continued.

CULTIVAR	SEASON	MEDIUM	GROWTH 0-5*	sd
NORGOLD RUSSET	WINTER	WILSON	3.1	0.8
NORGOLD RUSSET	WINTER	FAO	1.2	0.5
NORGOLD RUSSET	WINTER	WANG and HU	2.2	0.9
NORGOLD RUSSET	SPRING	WILSON	0	0
NORGOLD RUSSET	SPRING	FAO	4.4	1.0
NORGOLD RUSSET	SPRING	WANG and HU	3.0	0.6
YUKON GOLD	FALL	WILSON	3.4	1.2
YUKON GOLD	FALL	FAO	2.6	0.9
YUKON GOLD	FALL	WANG and HU	3.0	0.8
YUKON GOLD	WINTER	WILSON	2.8	0.8
YUKON GOLD	WINTER	FAO	1.0	1.0
YUKON GOLD	WINTER	WANG and HU	2.9	1.1

*GROWTH RATING

- 0 = dead
- 1 = alive but no development
- 2 = alive with 1 expanded leaf
- 3 = alive with 2 expanded leaves
- 4 = alive with more than 2 leaves but no visible shoot
- 5 = alive with more than 2 leaves and elongated shoot.

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Publications.

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